Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

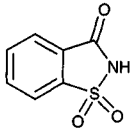
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 10.1

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Saccharin



Molecular formula: $C_7H_5NO_3S$
Molecular weight: 183.19
CAS Registry No.: 81-07-2, 6485-34-3 (Ca salt), 6381-91-5 (Ca salt hydrate), 128-44-9 (Na salt), 6155-57-3 (Na salt dihydrate)
Merck Index: 8463

SAMPLE
Matrix: beverage
Sample preparation: Sonicate 25 mL beverage for 15-20 min, filter (0.45 μm) if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 10:20:70:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: benzoic acid, hydroquinine, quinine

KEY WORDS
tonic water; soft drinks

REFERENCE
Valenti,L.P. Liquid chromatographic determination of quinine, hydroquinine, saccharin, and sodium benzoate in quinine beverages, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 782–784.

SAMPLE
Matrix: beverages, sweetener
Sample preparation: Sweetener. Dissolve 30 mg powdered tabletop sweetener in water and dilute to 25 mL, filter (0.2 μm PTFE). Beverages. Dilute fruit juice 1:10 with water. Degas carbonated beverages in a ultrasonic bath for 5 min, dilute 1:10 with water, filter. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 Dionex IonPak AG4A-SC
Column: 250 × 4 Dionex IonPak AS4A-SC
Mobile phase: Gradient. A was 1 mM sodium carbonate. B was 12.5 mM sodium carbonate. A:B 100:0 for 4.5 min, from 100:0 to 0:100 over 1 min, maintain at 0:100 for 22.5 min, from 0:100 to 100:0 over 0.1 min
Flow rate: 1
Injection volume: 50
Detector: UV 190 for 6 min, UV 206 22 min, then UV 190; Conductivity, Dionex ED40 in conductivity mode preceded by a Dionex ASRS-I suppressor (external water mode, 300 mA)

CHROMATOGRAM
Retention time: 18
Limit of detection: 19 ng/mL (UV), 260 ng/mL (conductivity)

OTHER SUBSTANCES
Simultaneous: acesulfame, aspartame

REFERENCE
Chen,Q.-C.; Mou,S.; Liu,K.; Yang,Z.; Ni,Z. Separation and determination of four artificial sweeteners and citric acid by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *771*, 135–143.

SAMPLE
Matrix: beverages, syrup
Sample preparation: Dilute syrup ten fold. Filter (0.45 μm) beverages and diluted syrup, inject a 10-20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:water 20:5:75
Flow rate: 2
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 30 ng

OTHER SUBSTANCES
Simultaneous: acesulfame, benzoic acid, caffeine, dulcin, p-hydroxybenzoic acid, vanillin

REFERENCE
Veerabhadrarao,M.; Narayan,M.S.; Kapur,O.; Sastry,C.S. Reverse phase liquid chromatographic determination of some food additives, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 578–582.

SAMPLE
Matrix: blood, feces, feed, milk, urine

Sample preparation: Condition a 3 mL quaternary amine SPE cartridge (J.T. Baker) with 3 mL MeOH, 3 mL concentrated ammonium hydroxide, and 3 mL 200 mM HCl. Feed, feces. Air dry feces at 50° for 17 h. 350 mg Feed or dried feces + 350 μL 100 mM NaOH, mix, add 2.5 mL 100 mM NaOH, mix, centrifuge at 500 g for 10 min, repeat extraction twice with 2.5 mL aliquots and once with a 2 mL aliquot of 100 mM NaOH. Combine the supernatants and make up to 10 mL with water. Remove a 500 μL aliquot and add it to the SPE cartridge, wash with two 1 mL aliquots of MeOH, wash with 2 mL water (add 3 μL 2-octanol to top of water as an anti-foaming agent), elute with two 2 mL aliquots of 200 mM pH 8.8 K_2HPO_4, add 80 μL phosphoric acid to the eluate, make up to 5 mL with water, inject an aliquot. Urine. 1 mL Urine + 8.5 mL 100 mM NaOH, make up to 10 mL with water. Remove a 500 μL aliquot and add it to the SPE cartridge, wash with two 1 mL aliquots of MeOH, wash with 2 mL water (add 3 μL 2-octanol to top of water as an anti-foaming agent), elute with two 2 mL aliquots of 200 mM pH 8.8 K_2HPO_4, add 80 μL phosphoric acid to the eluate, make up to 5 mL with water, inject an aliquot. Serum. 500 μL Serum + 4.25 mL 100 mM NaOH, make up to 5 mL with water. Remove a 500 μL aliquot and add it to the SPE cartridge, wash with two 1 mL aliquots of MeOH, wash with 2 mL water (add 3 μL 2-octanol to top of water as an anti-foaming agent), elute with two 2 mL aliquots of 200 mM pH 8.8 K_2HPO_4, add 80 μL phosphoric acid to the eluate, make up to 5 mL with water, inject an aliquot. Milk. Add 200 μL milk to the SPE cartridge, wash with two 1 mL aliquots of MeOH, wash with 2 mL water (add 3 μL 2-octanol to top of water as an anti-foaming agent), elute with two 2 mL aliquots of 200 mM pH 8.8 K_2HPO_4, add 80 μL phosphoric acid to the eluate, make up to 5 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:10 mM pH 2.2 KH_2PO_4 16:84
Column temperature: 30
Flow rate: 1
Detector: UV 254 or F ex 232 em 432

CHROMATOGRAM

Retention time: 1.9
Limit of quantitation: 2.45 pmole (F), 245 pmole (UV)

KEY WORDS

rat; SPE

REFERENCE

Tibbels,T.S.; Smith,R.A.; Cohen,S.M. Determination of saccharin in diet and biological materials, *J.Chromatogr.*, **1988**, *441*, 448–453.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 5.907

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: food
Sample preparation: 2 g Soy sauce or 1 g sugared fruit or roast beef + 10 g NaCl, make up to 50 mL with acetone, swirl vigorously, let stand for 30 min, filter, wash the solid with acetone, make up filtrate to 50 mL with acetone, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm monomeric C18 (Shoko, Kyoto)
Mobile phase: MeCN:50 mM pH 4.5 α-hydroxyisobutyric acid in water 22:34 containing 2.5 mM hexadecyltrimethylammonium bromide
Flow rate: 1
Injection volume: 20
Detector: UV 233

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Simultaneous: acesulfame-K, benzoic acid, 3-t-butyl-4-hydroxyanisole, butyl p-hydroxybenzoate, t-butylhydroxyquinone, dulcin, ethyl p-hydroxybenzoate, isobutyl p-hydroxybenzoate, isopropyl p-hydroxybenzoate, methyl p-hydroxybenzoate, sodium dehydroacetate, sorbic acid

KEY WORDS
soy sauce; roast beef; sugared fruit

REFERENCE
Chen,B.H.; Fu,S.C. Comparison of extraction methods and column types for the determination of additives by liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 625–643.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets and weigh out 1 g, add 1 mL formic acid, add 25 mL MeOH, shake mechanically for 10 min, make up to 50 mL with methanol. Remove 10 mL and centrifuge. 5 mL Supernatant + 5 mL 0.0025% p-hydroxybenzoic acid in MeOH:water 20:80, make up to 25 mL with water, inject an aliquot. (Analyze within 1 h.)

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb RP8
Mobile phase: MeOH:200 mM pH 3.5 phosphate buffer:water 20:10:70
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: p-hydroxybenzoic acid (18)

OTHER SUBSTANCES
Simultaneous: aspirin, p-aminophenol, 3-O-acetylascorbic acid, 2-O-acetylascorbic acid, Ascorbic acid, acetaminophen, O-acetyl-p-aminophenol, salicylic acid (UV 280), diacetyl-p-aminophenol (UV 280)

KEY WORDS
tablets

REFERENCE
Thomis,R.; Roets,E.; Hoogmartens,J. Analysis of tablets containing aspirin, acetaminophen, and ascorbic acid by high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 1830–1833.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, add 80 mL water, shake at 240 oscillations/min for 30 min, make up to 100 mL with water, mix well, centrifuge at 1500 rpm, dilute supernatant with water, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 3:97 (Buffer was 250 mM NaH_2PO_4 adjusted to pH 2.5 with phosphoric acid.) (Flush column with MeCN:water 5:95 at the end of each day.)
Flow rate: 2
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: pantothenic acid, pantoyllactone, panthenol

KEY WORDS
tablets

REFERENCE
Timmons,J.A.; Meyer,J.C.; Steible,D.J.; Assenza,S.P. Reverse phase liquid chromatographic assay for calcium pantothenate in multivitamin preparations and raw materials, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 510–513.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1 mL syrup to 50 mL with mobile phase, filter (0.45 μm), inject 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeCN:water:formic acid:methanesulfonic acid 500:500:1:1, pH adjusted to 3.5 with 10% NaOH
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: guaifenesin, dextromethorphan, benzoic acid

KEY WORDS
syrup

REFERENCE

Chen,T.M.; Pacifico,J.R.; Daly,R.E. High-pressure liquid chromatographic assay of dextromethorphan hydrobromide, guaifenesin, and sodium benzoate in an expectorant syrup, *J.Chromatogr.Sci.*, **1988**, *26*, 636–639.

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM

Retention time: 7.5

OTHER SUBSTANCES

Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, riboflavin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS

tablets

REFERENCE

Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam,

mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: yogurt

Sample preparation: 500 mg Homogenized yogurt + 7 mL buffer, sonicate for 2 min, shake mechanically for 20 min, centrifuge at 2000 rpm for 10 min, repeat extraction twice more. Combine the supernatants and make up to 25 mL with buffer. Remove a 5 mL extract and add it to 5 mL 10 mM tri-n-octylamine in chloroform, shake for 20 min, centrifuge at 2000 rpm for 10 min. Remove 2.5 mL of the organic phase and add it to 2.5 mL 100 mM sodium perchlorate in water, extract, centrifuge, inject an aliquot of the aqueous phase. (Buffer was 24.650 g $NaH_2PO_4.H_2O$ and 1.260 g $Na_2HPO_4.2H_2O$ made up to 2 L with water, pH 5.5.)

HPLC VARIABLES

Column: 250 × 4 10 μm RP-18 (Merck)

Mobile phase: MeOH:buffer 40:60 (Buffer was 900 μL 1 M phosphoric acid and 27.598 g $NaH_2PO_4.H_2O$ made up to 2 L with water, pH 4.5.)

Injection volume: 100

Detector: UV 270 for 4 min then UV 240

CHROMATOGRAM

Retention time: 2

Limit of detection: 20 μg/g

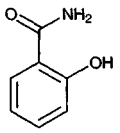
OTHER SUBSTANCES

Simultaneous: benzoic acid, sorbic acid

REFERENCE

Puttemans,M.L.; Branders,C.; Dryon,L.; Massart,D.L. Extraction of organic acids by ion-pair formation with tri-n-octylamine. Part 6. Determination of sorbic acid, benzoic acid, and saccharin in yogurt, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 80–82.

Salicylamide



Molecular formula: $C_7H_7NO_2$
Molecular weight: 137.14
CAS Registry No.: 65-45-2
Merck Index: 8480
Lednicer No.: 1 109

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Plasma. 200 μL Plasma + 100 μL 1 M perchloric acid, mix, centrifuge at 12000 g for 3 min, inject a 100 μL aliquot of the supernatant. Bile. Dilute bile 1:10 with water, inject a 20 μL aliquot. Urine. Filter (0.45 μm) urine, dilute with water 1:4, inject a 30 μL aliquot.

HPLC VARIABLES

Guard column: 40 μm μBondapak C18
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: 85 mM KH_2PO_4, pH adjusted to 3.35 with glacial acetic acid
Flow rate: 0.8 for 34 min, to 1.8 over 5 min, maintain at 1.8 for 11 min
Injection volume: 20-100
Detector: UV 313

CHROMATOGRAM

Retention time: 48
Limit of detection: 1.09 nmole

OTHER SUBSTANCES

Extracted: metabolites, conjugates

KEY WORDS

rat; plasma; whole blood

REFERENCE

Xu,X.; Pang,K.S. High-performance liquid chromatographic method for the quantitation of salicylamide and its metabolites in biological fluids, *J.Chromatogr.*, **1987**, *420*, 313–327.

SAMPLE

Matrix: blood
Sample preparation: 3 mL Whole blood + 6 mL ethyl acetate + 100 μL 10 μg/mL p-nitrophenol, shake, centrifuge at 3000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 40 μm μBondapak C18
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:0.5% acetic acid 40:60
Flow rate: 0.6
Injection volume: 20
Detector: UV 313

CHROMATOGRAM

Internal standard: p-nitrophenol
Limit of detection: 83 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS
rat; whole blood

REFERENCE
Xu,X.; Pang,K.S. High-performance liquid chromatographic method for the quantitation of salicylamide and its metabolites in biological fluids, *J.Chromatogr.*, **1987**, *420*, 313–327.

SAMPLE
Matrix: blood, urine
Sample preparation: Whole blood. Mix whole blood with 2 volumes cold MeOH, centrifuge, inject an aliquot of the supernatant. Urine. Dilute 24 h urine with 5 mL water, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 25 × 3.9 37-50 μm Bondapak C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: THF:1% acetic acid 5:95 containing 1.5 mM tetrabutylammonium hydroxide
Flow rate: 1.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Extracted: metabolites, conjugates

KEY WORDS
mouse; whole blood

REFERENCE
Howell,S.R.; Kotkoskie,L.A.; Dills,R.L.; Klaassen,C.D. 3-Hydroxylation of salicylamide in mice, *J.Pharm.Sci.*, **1988**, *77*, 309–313.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compounds in MeOH, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 3 μm Hitachi-Gel 3011 porous polymer (Hitachi)
Mobile phase: MeOH:ammonia 99:1
Flow rate: 0.03
Injection volume: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.16

OTHER SUBSTANCES
Also analyzed: acetaminophen, caffeine, bucetin (3-hydroxy-p-butyrophenetidine), phenacetin, dipyrone (sulpyrin), mefenamic acid, aspirin, salicylic acid, ethenzamide (o-ethoxybenzamide), theobromine, theophylline

KEY WORDS
semi-micro; porous polymer

REFERENCE
Matsushima,Y.; Nagata,Y.; Niyomura,M.; Takakusagi,K.; Takai,N. Analysis of antipyretics by semimicro liquid chromatography, *J.Chromatogr.*, **1985**, *332*, 269–273.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 30:1.5:0.5:68
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.19

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 230, 296

CHROMATOGRAM
Retention time: 2.4
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES
Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE
Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphet-

amine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in the mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 μm Lichrosorb RP 18
Mobile phase: MeOH:water 45:55 containing 1% acetic acid
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 4.20

OTHER SUBSTANCES

Simultaneous: acetaminophen, aspirin, phenacetin, salicylic acid

REFERENCE
Nivaud-Guernet,E.; Guernet,M.; Ivanovic,D.; Medenica,M. Effect of eluent pH on the ionic and molecular forms of the non-steroidal anti-inflammatory agents in reversed-phase high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2343–2357.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 8 μm Unisphere-PBD (polybutadiene on alumina) (Biotage, Charlottesville, VA)
Mobile phase: MeCN:water 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: acetaminophen, aspirin, phenacetin

REFERENCE
Jedrejewski,P.T.; Taylor,L.T. Comparison of silica-, alumina-, and polymer-based stationary phases for reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1995**, *33*, 438–445.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Cole-Parmer Model 4420) one hemisphere with 4 mL 100 mM pH 5.0 sodium phosphate buffer and p-nitrophenol at 1000 rpm for 2 min, add 5 mL ethyl acetate, vortex for 2 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 5 μm Partisil-5 ODS-3 RAC
Mobile phase: MeOH:25 mM KH_2PO_4:acetic acid 30:69:1
Flow rate: 2
Injection volume: 10
Detector: UV 313

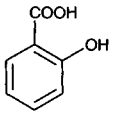
CHROMATOGRAM
Retention time: 7.5
Internal standard: p-nitrophenol (12.5)

OTHER SUBSTANCES
Extracted: gentisamide

KEY WORDS
rat; brain

REFERENCE
Morris,M.E.; Levy,G. Pharmacodynamics of the hypnotic effect of salicylamide in rats, *J.Pharm.Sci.*, **1985**, *74*, 599–602.

Salicylic acid



Molecular formula: $C_7H_6O_3$
Molecular weight: 138.12
CAS Registry No.: 69-72-7, 54-21-7 (Na salt)
Merck Index: 8484

SAMPLE

Matrix: blood
Sample preparation: Mix 100 μL plasma with 100 μL 2.5 μM IS, add 200 μL EtOH, vortex for 2 min, centrifuge at 1600 g for 15 min, dilute 50 μL of the supernatant with 950 μL mobile phase, filter (0.45 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 30 × 4.6 10 μm Spheri-10 RP18 (Alltech)
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:buffer 15:85 (Buffer was 30 mM sodium citrate and 30 mM sodium acetate, pH 5.45.)
Flow rate: 1
Injection volume: 50
Detector: UV 295

CHROMATOGRAM

Retention time: 7.2
Internal standard: 2.6-dihydroxybenzoic acid (11.4)
Limit of detection: 3.89 μM

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Coudray,C.; Mangournet,C.; Bouhadjeb,S.; Faure,H.; Favier,A. Rapid high-performance liquid chromatographic assay for salicylic acid in plasma without solvent extraction, *J.Chromatogr.Sci.*, **1996**, *34*, 166–173.

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 200 mM orthophosphoric acid to 250 μL chilled plasma within 10 min of centrifuging (if fresh plasma) or within 10 min of thawing (if frozen plasma), vortex for 20 s, centrifuge at 5800 g for 3 min. Inject a 200 μL aliquot onto column A and elute to waste with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 4.3 30 μm Hypersil C18 PEEK cartridge (Shandon, England); B 10 × 4 30 μm Hypersil C8 + 250 × 4.6 5 μm Nucleosil C8
Mobile phase: A Water:orthophosphoric acid 1000:1, pH 2.5; B MeCN:MeOH:water:orthophosphoric acid 150:200:650:1 (pH 2.6)
Flow rate: 1
Injection volume: 200
Detector: UV 225

CHROMATOGRAM

Retention time: 60
Limit of detection: 40 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: aspirin
Noninterfering: barbital, butobarbital, caffeine, 8-chlorotheophylline, clonazepam, cocaine, diazepam, flurazepam, furosemide, hydralazine, imipramine, nitrazepam, phenytoin, pindolol, propranolol, quinidine, theophylline
Interfering: xylazine, prazosin

KEY WORDS
column-switching; plasma

REFERENCE
McMahon,G.P.; Kelly,M.T. Determination of aspirin and salicylic acid in human plasma by column-switching liquid chromatography using on-line solid-phase extraction, *Anal.Chem.*, **1998**, *70*, 409–414.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with an equal volume of MeCN, vortex for 30 s, centrifuge at 900 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:water 22:5:73
Flow rate: 2.6
Injection volume: 100
Detector: UV 280

CHROMATOGRAM
Retention time: 7.0
Limit of quantitation: 5000 ng/mL

OTHER SUBSTANCES
Extracted: aspirin
Noninterfering: acetaminophen, albuterol, aminophylline, amitriptyline, amoxicillin, ampicillin, amobarbital, beclomethasone, carbamazepine, carbenicillin, chlordiazepoxide, cimetidine, clonazepam, cyproheptadine, debrisoquine, dextropropoxyphene, diazepam, digoxin, dihydroxyanthraquinone, ergotamine, ethosuximide, fluphenazine, furosemide, gentamicin, gentisic acid, guaifenesin, haloperidol, heparin, hydrocortisone, indomethacin, methdilazine, methyclothiazide, methylphenobarbitone, methysergide, metoclopramide, naproxen, nitrazepam, nystatin, penicillin, pentobarbitone, phenytoin, phenytoin, pizotifen, prazosin, prednisone, prochlorperazine, propranolol, spironolactone, sulfamethoxazole, theophylline, trifluoperazine, trimethoprim, valproic acid

KEY WORDS
plasma

REFERENCE
Cham,B.E.; Ross-Lee,L.; Bochner,F.; Imhoff,D.M. Measurement and pharmacokinetics of acetylsalicylic acid by a novel high performance liquid chromatographic assay, *Ther.Drug Monit.*, **1980**, *2*, 365–372.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3

Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 8.0
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS
serum

REFERENCE
Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 15 μg/mL β-hydroxyethyltheophylline in MeCN, mix for 30 s, centrifuge at 13000 g for 5 min. Inject the supernatant (about 20 μL).

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:buffer 9.75:90.25 (Buffer was 100 mM KH_2PO_4 adjusted to pH 4.0 with phosphoric acid.) (At the end of each day clean with water for 20 min and MeOH for 30 min.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Internal standard: β-hydroxyethyltheophylline (5.8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: aspirin
Simultaneous: dyphylline, theophylline, caffeine, acetaminophen, procainamide, N-acetylprocainamide
Noninterfering: benzoic acid

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Frawley,V.L. Theophylline, dyphylline, caffeine, acetaminophen, salicylate, acetylsalicylate, procainamide, and N-acetylprocainamide determined in serum with a single liquid-chromatographic assay, *Clin.Chem.*, **1982**, *28*, 2157–2160.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1-2 mL Plasma + 600-1000 mg potassium carbonate plasma + 1 mL MeOH, shake for 1 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 25-50 μL aliquot.

HPLC VARIABLES
Column: 610 × 2 25-50 μm Bondapak AX/Corasil anion exchange
Mobile phase: MeOH:pH 5.6 phosphate buffer 15:85
Flow rate: 0.5
Injection volume: 10-50
Detector: UV 254, 280

CHROMATOGRAM
Retention time: 11.2 (at UV 280)
Internal standard: sodium salicylate

OTHER SUBSTANCES
Extracted: dipyrone (at UV 254)

KEY WORDS
plasma; sodium salicylate is IS

REFERENCE
Asmardi,G.; Jamali,F. High-performance liquid chromatography of dipyrone and its active metabolite in biological fluids, *J.Chromatogr.*, **1983**, *277*, 183–189.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Whole blood + 1 mL 1 M HCl + 200 μL 1 mg/mL β-hydroxyethyltheophylline in MeOH + 10 mL dichloromethane, rotate for 10 min, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS (Altex)
Mobile phase: MeOH:water:glacial acetic acid 40:66:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 6.4
Internal standard: β-hydroxyethyltheophylline (2)
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: methyl salicylate, theophylline
Noninterfering: acetaminophen, amobarbital, butalbital, caffeine, carbamazepine, glutethimide, ibuprofen, indomethacin, meprobamate, methaqualone, pentobarbital, phenobarbital, phenylbutazone, phenytoin, primidone, secobarbital

KEY WORDS
whole blood

REFERENCE
Levine,B.; Caplan,Y.H. Liquid chromatographic determination of salicylate and methyl salicylate in blood and application to a postmortem case, *J.Anal.Toxicol.*, **1984**, *8*, 239–241.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 0.2 mL 1 M HCl + 10 mL diethyl ether, gently mix for 10 min, centrifuge at 1500 rpm for 4 min. Remove the organic phase, evaporate it to dryness at 0° under a stream of nitrogen, add 200 μL mobile phase, vortex 90 s, inject a 5-100 μL aliquot.

HPLC VARIABLES
Guard column: 23 × 3.9 μBondapak C18/Porasil B

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:1-butanol:orthophosphoric acid 270:720:10:0.13
Column temperature: 47
Flow rate: 1.8
Injection volume: 5-100
Detector: UV 234

CHROMATOGRAM
Retention time: 8.0
Internal standard: m-anisic acid (9.6)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: aspirin
Noninterfering: acetaminophen, albuterol, aminophylline, amitriptyline, atenolol, beclomethasone, bromazepam, caffeine, carbamazepine, chloral hydrate, chlordiazepoxide, cimetidine, clonazepam, codeine, desipramine, dexamethasone, dextropropoxyphene, diazepam, dicyclomine, digoxin, disopyramide, doxycycline, ergotamine, ethosuximide, furosemide, gentisic acid, haloperidol, hydrocortisone, imipramine, indomethacin, levodopa, lignocaine, lithium carbonate, meperidine, methdilazine, methylphenobarbitone, methylprednisolone, methysergide, metoclopramide, metoprolol, mexiletine, midazolam, naphthoxyacetic acid, nitrazepam, nitroglycerin, nortripyline, oxazepam, oxpranolol, pentobarbitone, pethidine, phenytoin, prednisolone, prednisone, primidone, procainamide, prochlorperazine, propranolol, quinidine, salicyluric acid, spironolactone, sulfamethoxazole, theophylline, trimethoprim, valproic acid, verapamil, warfarin
Interfering: methyclothiazide

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Brandon,R.A.; Eadie,M.J.; Smith,M.T. A sensitive liquid chromatographic assay for plasma aspirin and salicylate concentrations after low doses of aspirin, *Ther.Drug Monit.*, **1985**, *7*, 216–221.

SAMPLE
Matrix: blood
Sample preparation: Activate a 1 mL Bond-Elut C8 SPE cartridge with 2 mL MeOH then 1 mL 10 mM HCl, do not allow it to dry completely. Sonicate 1 mL whole blood for 20-30 min then apply to cartridge. Wash with 100 μL water, elute with three 500 μL portions of MeOH:MeCN:1% aqueous ammonium hydroxide 50:20:30, combine eluents and evaporate to dryness under a stream of nitrogen at 40°. Redissolve in 1 mL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Spherisorb ODS
Mobile phase: MeCN:MeOH:buffer 35:13:52 (Buffer was water adjusted to pH 3.2 with orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: ketoprofen, acetaminophen, naproxen, fenoprofen, ibuprofen, indomethacin

KEY WORDS
whole blood; SPE

REFERENCE
Moore,C.M.; Tebbett,I.R. Rapid extraction of anti-inflammatory drugs in whole blood for HPLC analysis, *Forensic Sci.Int.*, **1987**, *34*, 155–158.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Blood + 1 mL water + 50 μL 76 mg/L allobarbital in EtOH:water 10:90 + 5 mL ethyl acetate, shake by hand, add 2 mL of 0.1 M HCl. Mix by inversion with a mechanical shaker for 5 min. Centrifuge at 2700 rpm for 5-10 min. Remove ethyl acetate and evaporate to dryness under a stream of nitrogen at room temperature. Take up in 200 μL MeOH, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: Guard-Pak precolumn insert
Column: 200 × 4.6 5 μm Hypersil octadecylsilane
Mobile phase: Gradient. MeCN:1 mM pH 3.2 phosphate buffer from 20:80 to 40:60 over 10 min, stay at 40:60 for 6 min, to 20:80 over 4 min
Column temperature: 60
Flow rate: 3
Injection volume: 20
Detector: UV 202

CHROMATOGRAM
Retention time: 3.6
Internal standard: allobarbital (2.9)
Limit of quantitation: 10000 ng/mL pharmacokinetics

OTHER SUBSTANCES
Extracted: butalbital
Simultaneous: caffeine, aspirin

REFERENCE
Drost,M.L.; Walter,L. Blood and plasma concentrations of butalbital following single oral doses in man, *J.Anal.Toxicol.*, **1988**, *12*, 322–324.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 400 μL 100 μg/mL 8-chlorotheophylline in MeCN, vortex for 10 s, centrifuge at 15000 rpm for 3 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water:glacial acetic acid 4:84:12 containing 4.84 g/L Trizma, pH 2.3
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.26
Internal standard: 8-chlorotheophylline (5.29)
Limit of quantitation: 8 μg/mL

OTHER SUBSTANCES
Extracted: acetaminophen, theophylline
Simultaneous: caffeine, cefazolin, cimetidine, ergotamine, glutethimide, heparin, methamphetamine, propranolol, sulfamethoxazole, theobromine, tobutamide, trimethoprim
Noninterfering: amitriptyline, amobarbital, ampicillin, butabarbital, butalbital, celbenine, chlordiazepoxide, chlorpromazine, clorazepate, desipramine, diazepam, doxepin, ethchlorvynol, fluphenazine, hydroxyzine, ibuprofen, imipramine, isoniazid, lidocaine, mephobarbital, mesoridazine, methaqualone, methyluric acid, naprotyline, nordiazepam, nortriptyline, oxazepam, pentobarbital, perphenazine, phenelzine, phenmetrazine, phenobarbital, phenylbutazone, phenytoin, prednisolone, prednisone, procainamide, prochlorperazine, promazine, promethazine, propoxyphene, protriptyline, pyrilamine, secobarbital, thioridazine, thiothixene, timolol, trazodone, triazolam, trifluoperazine

KEY WORDS
serum

REFERENCE
Osterloh,J.; Yu,S. Simultaneous ion-pair and partition liquid chromatography of acetaminophen, theophylline and salicylate with application to 500 toxicologic specimens, *Clin.Chim.Acta*, **1988**, *175*, 239–248.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 2.5 mL 10 μL/mL salicylamide in MeOH, stir, centrifuge. Remove a 1.8 mL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:50 mM acetic acid 40:60
Flow rate: 1
Detector: UV 237

CHROMATOGRAM
Retention time: 5.6
Internal standard: salicylamide (7.7)
Limit of detection: 500 ng/mL

KEY WORDS
plasma; rat; dog; pharmacokinetics; also for humans (see Int.J.Clin.Pharmacol.Ther.Toxicol. 1988, 26, 421)

REFERENCE
Ramis,J.; Mis,R.; Forn,J. Pharmacokinetics of fosfosal in rats and dogs, *Arzneimittelforschung*, **1989**, *39*, 74–77.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL anisic acid + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:0.5 mM phosphoric acid 30:70
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Internal standard: anisic acid

KEY WORDS
plasma; rat

REFERENCE
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE
Matrix: blood

Sample preparation: Add o-anisic acid to 1 mL plasma, acidify with HCl, extract with diethyl ether. Remove the organic layer and evaporate it to dryness, reconstitute the residue, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-8
Mobile phase: MeCN:20 mM phosphoric acid 15:85
Flow rate: 1.6
Detector: UV 237

CHROMATOGRAM
Internal standard: o-anisic acid
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: aspirin

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Benedek,I.H.; Joshi,A.S.; Pieniaszek,H.J.; King,S.-Y.P.; Kornhauser,D.M. Variability in the pharmacokinetics and pharmacodynamics of low dose aspirin in healthy male volunteers, *J.Clin.Pharmacol.*, **1995**, *35*, 1181–1186.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.22 μm), inject a 2 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: MeCN:50 mM pH 3.0 phosphate buffer 1.5:98.5
Flow rate: 0.5
Injection volume: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Extracted: aspirin

KEY WORDS
serum; direct injection

REFERENCE
Nimura,N.; Itoh,H.; Kinoshita,T. Diol-bonded silica gel as a restricted access packing forming a binary-layered phase for direct injection of serum for the determination of drugs, *J.Chromatogr.A*, **1995**, *689*, 203–210.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 200 μL Plasma + 200 μL 5 μg/mL IS in 200 mM HCL:200 mM orthophosphoric acid 50:50, vortex for 1-2 s, add 400 μL MeCN, vortex, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 100-120 mg NaCl, vortex briefly, let stand at 4° for 10 min, vortex, centrifuge at 10500 g for 1 min, inject a 10 μL aliquot of the upper organic layer. Whole blood. 200 μL Lysed whole blood + 400 μL 5 μg/mL IS in 200 mM HCL:200 mM orthophosphoric acid 50:50, vortex for 1-2 s, add 600 μL MeCN, vortex, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 200 mg NaCl, vortex briefly, let stand at 4° for 10 min, vortex, centrifuge at 10500 g for 1 min, inject a 10 μL aliquot of the upper organic layer.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:water:85% orthophosphoric acid 18:74:0.09 (Before use prime column by recycling 200 mL mobile phase + 400 μL di-n-butylamine overnight at 0.3 mL/min.)
Column temperature: 30
Flow rate: 1
Injection volume: 10
Detector: UV 237

CHROMATOGRAM

Retention time: 6.8
Internal standard: 2-methylbenzoic acid (8.9)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, aspirin, gentisic acid, salicyluric acid

KEY WORDS

plasma; whole blood; pharmacokinetics

REFERENCE

Kees,F.; Jehnich,D.; Grobecker,H. Simultaneous determination of acetylsalicylic acid and salicylic acid in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *677*, 172–177.

SAMPLE

Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 5.2
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, luminal contents, tissue
Sample preparation: Homogenize intestinal tissue with water, make up to 25 mL with water, sonicate for 20 min. 100 μL Whole blood, luminal contents or tissue homogenate + 200 μL MeCN + 100 μL 5 mM HCl, vortex for 30 s, centrifuge at 3000 rpm for 5 min, inject a 30 μL aliquot of the clear supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:glacial acetic acid 35:65:4
Flow rate: 1.5
Injection volume: 30
Detector: UV 313

CHROMATOGRAM
Limit of detection: <1 μg/mL

KEY WORDS
rat; whole blood; intestine; pharmacokinetics

REFERENCE
Choi,Y.M.; Chung,S.M.; Chiou,W.L. First-pass accumulation of salicylic acid in gut tissue after absorption in anesthetized rat, *Pharm.Res.*, **1995**, *12*, 1323–1327.

SAMPLE
Matrix: blood, plants, urine, water
Sample preparation: Urine, plasma, water. Acidify 2 mL urine, plasma, or drinking water to pH 1 with 2 M HCl, shake with two 5 mL aliquots of diethyl ether, centrifuge. Remove the organic layer and add it to a few drops of saturated sodium bicarbonate solution, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL MeOH, inject a 25 μL aliquot. Hay, grain. Homogenize 30 g hay or grain with 10 mL 10% NaOH and 290 mL water, heat on a steam bath for 1 h, filter. Acidify a 10 mL aliquot of the filtrate to pH 1 with 2 M HCl, shake with two 5 mL aliquots of diethyl ether, centrifuge. Remove the organic layer and add it to a few drops of saturated sodium bicarbonate solution, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18 radial compression
Mobile phase: MeCN:0.05% phosphoric acid 30:70 (plasma) or MeCN:0.2% acetic acid 20:80 (urine)
Flow rate: 1.5
Injection volume: 25
Detector: F ex 313 em 425

CHROMATOGRAM
Limit of detection: 100 ng/mL (plasma), 5 μg/mL (urine)

KEY WORDS
horse; plasma; hay; grain; pharmacokinetics

REFERENCE
Beaumier,P.M.; Fenwick,J.D.; Stevenson,A.J.; Weber,M.P.; Young,L.M. Presence of salicylic acid in standardbred horse urine and plasma after various feed and drug administrations, *Equine.Vet.J.*, **1987**, *19*, 207–213.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 900 μL 270 mM HCl + 100 μL 100 μg/mL α-phenylcinnamic acid in MeOH + 10 mL dichloromethane, shake at 125 cycles/min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot. Urine. 2 mL Urine + 900 μL 270 mM HCl + 100 μL 100 μg/mL α-phenylcinnamic acid in MeOH + 10 mL hexane, shake at 125 cycles/

min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:1% acetic acid 60:40
Flow rate: 2
Injection volume: 25
Detector: UV 300

CHROMATOGRAM
Retention time: 3.0
Internal standard: α-phenylcinnamic acid (8.0)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: aspirin (UV 280), salsalate

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Harrison,L.I.; Funk,M.L.; Ober,R.E. High-pressure liquid chromatographic determination of salicylsalicylic acid, aspirin, and salicylic acid in human plasma and urine, *J.Pharm.Sci.*, **1980**, *69*, 1268–1271.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 200 μL 330 mM perchloric acid, mix thoroughly, centrifuge at 2600 g for 10 min, inject an aliquot of the supernatant. Urine. 100 μL Urine + 900 μL 200 mM pH 5 phosphate buffer, mix, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 8 μm Cp Spher C8 (Chrompack) + 250 × 4.6 5 μm Spherisorb 5 ODS
Mobile phase: Gradient. MeCN:buffer from 1:99 to 35:65 over 35 min, return to initial conditions over 3 min, re-equilibrate for 2 min. (Buffer was 6 g orthophosphoric acid and 1 mL glacial acetic acid in 1 L distilled water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 236

CHROMATOGRAM
Retention time: 32.09
Limit of quantitation: 5 μg/mL (urine), 200 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites, gentisic acid, hippuric acid, salicylic acid acyl glucuronide, salicylic acid phenolic glucuronide, salicyluric acid, salicyluric acid phenolic glucuronide

KEY WORDS
pharmacokinetics

REFERENCE
Vree,T.B.; van Ewijk-Beneken Kolmer,E.W.J.; Verwey-van Wissen,C.P.W.G.M.; Hekster,Y.A. Direct gradient reversed-phase high-performance liquid chromatographic determination of salicylic acid, with the corresponding glycine and glucuronide conjugates in human plasma and urine, *J.Chromatogr.B*, **1994**, *652*, 161–170.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Adjust pH of plasma to 3 with 43% phosphoric acid. 500 μL Acidified plasma + 300 μL 10 μg/mL m-hydroxybenzoic acid in MeCN + 1.2 mL MeCN, vortex

for 1-2 min, centrifuge at 2000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 500 μL mobile phase, centrifuge at 1500 g for 2 min, inject a 50 μL aliquot of the supernatant. Urine. Dilute 25 μL urine to 500 μL with 40 μg/mL m-hydroxybenzoic acid in MeCN:water 10:90, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 (Brownlee)
Column: 150 × 4.6 5 μm Axxiom ODS (Springfield VA)
Mobile phase: MeCN:MeOH:25 mM acetic acid 8.5:8.5:83
Flow rate: 1
Injection volume: 25-50
Detector: UV 310

CHROMATOGRAM
Retention time: 16.5
Internal standard: m-hydroxybenzoic acid (8.2)
Limit of quantitation: 5 μg/mL (urine), 100 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: etodolac, ibuprofen, ketorolac

KEY WORDS
plasma; paper also contains details of preparative HPLC; pharmacokinetics

REFERENCE
Liu,J.-H.; Smith,P.C. Direct analysis of salicylic acid, salicyl acyl glucuronide, salicyluric acid and gentisic acid in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 61–70.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 12.12

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in dilute HCl (pH 2), sonicate if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil-BDS C18
Mobile phase: MeOH:THF:buffer 11:4:85 (Buffer was 8.6 g $NaH_2PO_4.H_2O$, 8.2 g NaCl, 2.2 g sodium 1-heptanesulfonate, and 5.75 mL 85% phosphoric acid in 2 L water, pH 2.0.)
Column temperature: 35
Flow rate: 1.5
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 38

OTHER SUBSTANCES
Simultaneous: impurities, 5-aminosalicylic acid

KEY WORDS
impurity in 5-aminosalicylic acid

REFERENCE
Kersten,B.S.; Catalano,T.; Rozenman,Y. Ion-pairing high-performance liquid chromatographic method for the determination of 5-aminosalicylic acid and related impurities in bulk chemical, *J.Chromatogr.*, **1991**, *588*, 187–193.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 20 mg/mL solution of bulk aspirin in dichloromethane, inject a 10 μL aliquot as soon as dissolution is complete. Tablets. Prepare a 20 mg/mL solution of ground aspirin tablets in dichloromethane, filter (0.45 μm) immediately, immediately inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 6 μm Zorbax SIL
Mobile phase: Hexane:chloroform:acetic acid 80:19:3 (Before first use pump 10 column volumes of dichloromethane:acetic acid:2,3-dimethoxypropane 96:2:2 through column at 3 mL/min.)
Flow rate: 3
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 1.6
Limit of detection: 14 ppm

OTHER SUBSTANCES
Simultaneous: aspirin, salsalate

KEY WORDS
normal phase; tablets

REFERENCE
Pfeiffer,C.D.; Pankey,J.W. Determination of related compounds in aspirin by liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 511–514.

SAMPLE
Matrix: formulations
Sample preparation: Vaccine. Centrifuge vaccine at 3400 g for 15 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 5 × 4 5 μm Hypersil C18
Column: 210 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:water:orthophosphoric acid 35:35:0.9, pH 2.5
Flow rate: 0.6
Injection volume: 25
Detector: UV 222

CHROMATOGRAM
Retention time: 6.5
Internal standard: salicylic acid

OTHER SUBSTANCES
Simultaneous: dithiosalicylic acid, thimerosal, thiosalicylic acid,

KEY WORDS
vaccine; salicylic acid is IS

REFERENCE
Tleugabulova,D.; Gonzalez Perez,I. Reversed-phase high-performance liquid chromatographic study of thimerosal stability in Cuban recombinant hepatitis B vaccine, *J.Chromatogr.A*, **1996**, *729*, 219–227.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate 75 mg powdered tablets with 25 mL mobile phase for 15 min, filter (paper), inject a 135 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Scharlau Science, Spain)
Mobile phase: MeOH:20 mM pH 4.0 KH_2PO_4 30:70 adjusted to pH 4.0 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 135
Detector: UV 224

CHROMATOGRAM
Retention time: 9.7
Limit of quantitation: 1.5 mg/L

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, thiamine

KEY WORDS
tablets

REFERENCE
Gámiz-Gracia,L.; Luque de Castro,M.D. An HPLC method for the determination of vitamin B1, caffeine, acetylsalicylic acid, and the impurities of salicylic acid in a pharmaceutical preparation, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2123–2133.

SAMPLE
Matrix: formulations
Sample preparation: Place 5 tablets in MeCN:MeOH:85% phosphoric acid 92:8:0.5, sonicate 15 min, shake 15 min, dilute to 250 mL. Centrifuge an aliquot in 50 mL tube at 2000 rpm for 15 min and filter supernatant (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve (Waters)
Mobile phase: MeCN:water:85% phosphoric acid 24:76:0.5
Column temperature: 35
Flow rate: 2
Injection volume: 10
Detector: UV 295

CHROMATOGRAM
Retention time: 3.3

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, acetaminophen

KEY WORDS
film coated tablets; tablets

REFERENCE
Fogel,J.; Epstein,P.; Chen,P. Simultaneous high-performance liquid chromatography assay of acetylsalicylic acid and salicylic acid in film-coated aspirin tablets, *J.Chromatogr.*, **1984**, *317*, 507–511.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder and add about 250 mg aspirin to 100 mL chloroform saturated with citric acid containing 500 μL formic acid, add 500 mg solid citric acid, sonicate for 2 min, centrifuge or filter, inject an aliquot. (If buffers or antacid are present add ground tablets equivalent to about 500 mg aspirin to 3 g acid-washed siliceous earth, mix, add 2 mL 6 M HCl, mix, add to a 200 × 25 column, dry wash container with siliceous earth, add to column, elute column with chloroform saturated with citric acid at 10 mL/min. Collect 150 mL eluent, add 1 mL formic acid, make up to 200 mL with chloroform saturated with citric acid, add 500 mg citric acid, shake, inject an aliquot.)

HPLC VARIABLES
Column: 250 × 4.6 5μm Zorbax-Sil
Mobile phase: Chloroform:methylene chloride:acetonitrile:formic acid 700:300:30:4 (At the end of the day wash the column with 200 mL MeOH.)
Flow rate: 2
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: aspirin, acetylsalicylsalicylic acid, acetylsalicylic acid anhydride, excipients

KEY WORDS
normal phase; tablets; SPE

REFERENCE
Galante,R.N.; Visalli,A.J.; Grim,W.M. Stabilized normal-phase high-performance liquid chromatographic analysis of aspirin and salicylic acid in solid pharmaceutical dosage forms, *J.Pharm.Sci.*, **1984**, *73*, 195–197.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets and weigh out 1 g, add 1 mL formic acid, add 25 mL MeOH, shake mechanically for 10 min, make up to 50 mL with methanol. Remove 10 mL and centrifuge. 5 mL Supernatant + 5 mL 0.0025% p-hydroxybenzoic acid in MeOH:water 20:80, make up to 25 mL with water, inject an aliquot. (Analyze within 1 h.)

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb RP8
Mobile phase: MeOH:200 mM pH 3.5 phosphate buffer:water 20:10:70
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 3
Internal standard: p-hydroxybenzoic acid (18)

OTHER SUBSTANCES
Simultaneous: aspirin (UV 254), p-aminophenol (UV 254), 3-O-acetylascorbic acid (UV 254), 2-O-acetylascorbic acid (UV 254), saccharin (UV 254), acetaminophen (UV 254), O-acetyl-p-aminophenol (UV 254), Ascorbic acid (UV 254), diacetyl-p-aminophenol

KEY WORDS
tablets

REFERENCE
Thomis,R.; Roets,E.; Hoogmartens,J. Analysis of tablets containing aspirin, acetaminophen, and ascorbic acid by high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 1830–1833.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: phenyl
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM KH_2PO_4 containing 0.52% tetrabutylammonium phosphate, pH adjusted to 7.1 with KOH.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 6
Internal standard: salicylic acid

OTHER SUBSTANCES
Simultaneous: nitroprusside

KEY WORDS
injections; 5% dextrose; salicylic acid is IS

REFERENCE
Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

SAMPLE
Matrix: formulations
Sample preparation: Condition a 500 mg Extract Clean silica SPE cartridge (Alltech stock no. 209250) with 2 mL hexane. Allow a solution of aspirin in 10 mM sorbitan trioleate in CFC-11 to evaporate, dissolve the residue in 5 mL hexane. Add 1 mL to the SPE cartridge, elute with two 2 mL portions of mobile phase, make up eluate to 5 mL with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 3040 μm Perisorb RP-8 Pellicular (Upchurch)
Column: 250 × 4.6 5 μm Econosphere C8
Mobile phase: MeOH:THF:1 M phosphoric acid:water 44:5:5:46
Flow rate: 1

Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 10.6

OTHER SUBSTANCES
Extracted: aspirin, degradation products

KEY WORDS
aerosols; SPE

REFERENCE
Blondino,F.E.; Byron,P.R. The quantitative determination of aspirin and its degradation products in a model solution aerosol, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 111–119.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out amount containing 100 mg salicylic acid, make up to 50 mL with mobile phase. Remove a 2 mL aliquot and dilute it to 10 mL with mobile phase, filter, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water:96% acetic acid 55:44:1, pH 3.0
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.44

OTHER SUBSTANCES
Simultaneous: triamcinolone acetonide

KEY WORDS
topical solution

REFERENCE
Kedor-Hackmann,E.R.M.; Gianotto,E.A.S.; Santoro,M.I.R.M. Determination of triamcinolone acetonide and salicylic acid in pharmaceutical formulations by high performance liquid chromatography, *Pharmazie*, **1996**, *51*, 63–63.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: Lichrospher 100 RP-18
Mobile phase: MeOH:water containing 10 mM citric acid 60:40
Column temperature: 45
Flow rate: 1
Detector: F ex 300 em 408

KEY WORDS
rat; intestine; Caco-2 monolayer

REFERENCE
Takagi,M.; Taki,Y.; Sakane,T.; Nadai,T.; Sezaki,H.; Oku,N.; Yamashita,S. A new interpretation of salicylic acid transport across the lipid bilayer: Implication of pH-dependent but not carrier-mediated absorption from the gastrointestinal tract, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 1175–1180.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5μm Brownlee C18 (Applied Biosystems)
Mobile phase: MeCN:0.03% phosphoric acid:triethylamine 64:35:1, pH 2.0
Flow rate: 1
Injection volume: 20
Detector: UV 237

OTHER SUBSTANCES
Simultaneous: diflunisal

REFERENCE
Hung,D.Y.; Mellick,G.D.; Anissimov,Y.G.; Weiss,M.; Roberts,M.S. Hepatic disposition and metabolite kinetics of a homologous series of diflunisal esters, *J.Pharm.Sci.*, **1998**, *87*, 943–951.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeOH:50 mM phosphoric acid 25:75
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: indoleacetic acid, phenylacetic acid

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:8 mM pH 7.0 phosphate buffer 5:95
Flow rate: 1.2
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: lodoxamide

KEY WORDS
aerosol

REFERENCE
Havel,H.A.; Beaubien,L.J.; Haaland,P.D. Analysis of the variance components in a pharmaceutical aerosol product: lodoxamide tromethamine, *J.Pharm.Sci.*, **1985**, *74*, 978–982.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compounds in MeOH, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 3 μm Hitachi-Gel 3011 porous polymer (Hitachi)
Mobile phase: MeOH:ammonia 99:1
Flow rate: 0.03
Injection volume: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.56

OTHER SUBSTANCES
Also analyzed: acetaminophen, caffeine, bucetin (3-hydroxy-p-butyrophenetidine), phenacetin, dipyrone (sulpyrin), mefenamic acid, aspirin, salicylamide, ethenzamide (o-ethoxybenzamide), theobromine, theophylline

KEY WORDS
semi-micro; porous polymer

REFERENCE
Matsushima,Y.; Nagata,Y.; Niyomura,M.; Takakusagi,K.; Takai,N. Analysis of antipyretics by semimicro liquid chromatography, *J.Chromatogr.*, **1985**, *332*, 269–273.

SAMPLE
Matrix: solutions
Sample preparation: Add 500 μL of a solution in MeCN to 100 mg finely powdered potassium carbonate, add 250 μL 3.8 mM 18-crown-6 in MeCN, add 250 μL 0.8 mM reagent in MeCN, heat at 80° in the dark for 20 min, cool, inject a 5 μL aliquot. (Synthesize the reagent, 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone, as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane:ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°).)

HPLC VARIABLES
Column: 100 × 4 10 μm Radial-Pak C18 (Waters)
Mobile phase: Gradient. MeOH:water from 57:43 to 100:0 over 20 min, maintain at 100:0 for 12 min
Flow rate: 2
Injection volume: 5
Detector: F ex 370 em 450

CHROMATOGRAM
Retention time: 7.1
Limit of detection: 0.3-1 fmole

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, arachidic acid, arachidonic acid, benzoic acid, butyric acid, capric acid, caproic acid, caprylic acid, deoxyuridine, glucuronic acid, imidazole-4-acetic acid, lauric acid, linoleic acid, linolenic acid, margaric acid, 1-methyl-4-imidazoleacetic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, propionic acid, stearic acid, thymidine, uridine, valeric acid

KEY WORDS
derivatization

REFERENCE
Yamaguchi,M.; Hara,S.; Matsunaga,R.; Nakamura,M.; Ohkura,Y. 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as a new fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *346*, 227–236.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 35:1.5:0.5:63
Flow rate: 1.5
Injection volume: 10
Detector: UV 261

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: physostigmine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: React the carboxylic acid, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in MeCN at 45° for 2 h, inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH satu-

rated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.6 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6
Limit of detection: 1 pmole

OTHER SUBSTANCES
Simultaneous: benzoic acid, quinoxaline-2-carboxylic acid

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: 10 μL Solution + 500 μL micelle solution + 25 μL 28 mg/mL 9-bromomethylacridine in acetone, mix, heat at 60° for 6 min, inject a 20 μL aliquot. (Micelle solution was 25 mM Arkopal N-130 (a polyoxyethylene(13)nonylphenol, Hoechst Holland, Amsterdam) in 10 mM pH 7.0 phosphate buffer containing 6 mM tetrakis(decyl)ammonium bromide. Synthesize 9-bromomethylacridine as follows. Heat 10 g diphenylamine, 10 mL glacial acetic acid, and 40 g anhydrous zinc chloride to 220°, evaporate excess acetic acid with stirring, heat at 220-230° for 6 h, digest with hot 10% sulfuric acid, make strongly alkaline with 25% ammonia to dissolve the zinc chloride. Extract the insoluble residue with toluene. Extract the organic layer with 10% sulfuric acid, make the aqueous layer alkaline with aqueous ammonia. Collect the yellow precipitate that separates and recrystallize it twice from petroleum ether to give 9-methyl acridine as pale yellow needles. Reflux 560 mg 9-methylacridine, 445 mg N-bromosuccinimide, and 10 mg benzoyl peroxide in 30 mL carbon tetrachloride for more than 2 h, cool, chromatograph on silica gel with benzene:ethyl acetate 30:1 (Caution! Benzene is a carcinogen!) to obtain 9-bromomethylacridine as yellow crystals (mp 147-151°) (Anal. Lett. 1987, 20, 1581).)

HPLC VARIABLES
Guard column: 10 × 2.1 40 μm Chromsep C18 (Chrompack)
Column: 100 × 3 5 μm Chromspher C18 (Chrompack)
Mobile phase: Gradient. MeOH:water from 20:80 to 100:0 over 13 min.
Injection volume: 20
Detector: UV 254, F ex 362 em 418

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: cholic acid, ibuprofen, valproic acid

KEY WORDS
derivatization

REFERENCE
van der Horst,F.A.L.; Post,M.H.; Holthuis,J.J.M.; Brinkman,U.A.T. Derivatization of carboxylic acids with 9-bromomethylacridine using micellar phase-transfer catalysis, *Chromatographia*, **1989**, *28*, 267–273.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 16.5

OTHER SUBSTANCES
Simultaneous: acetophenone, amphetamine, desipramine, ethylmorphine, imipramine, mefenamic acid, methamphetamine, morphine, phenylbutazone

KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spheri-5 RP-8
Mobile phase: MeOH:buffer 30:70 (Prepare buffer by mixing 4 mM Na_2HPO_4 and 7 mM KH_2PO_4 to achieve pH 7.)
Flow rate: 1
Injection volume: 50
Detector: F ex 355 em 460 (408 nm cutoff filter) following post-column extraction. The column effluent mixed with 50 μg/mL reagent in mobile phase pumped at 0.5 mL/min and then with chloroform pumped at 1 mL/min and the mixture flowed through a 1.8 m × 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylene-backed 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector. (Synthesize the reagent, α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate, as follows. Stir 20 mmoles 3,4-dimethoxyphenylacetonitrile and 20 mmoles p-toluamide in 50 mL EtOH at 50°, add 5 mL 50% aqueous KOH slowly, stir at 50° for 5 min, cool to room temperature, filter, dry the precipitate of α-(3,4-dimethoxyphenyl)-4'- methylcinnamonitrile. Dissolve 20 mmoles α-(3,4-dimethoxyphenyl)-4'-methylcinnamonitrile, 20 mmoles N-bromosuccinimide, and 20 mg benzoyl peroxide in 100 mL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), reflux with stirring for 1.5 h , cool, filter, evaporate to dryness under reduced pressure, recrystallize from MeOH to give α-(3,4-dimethoxyphenyl)-4'-bromomethylcinnamonitrile. Vigorously stir 30 mmoles anhydrous dimethylamine in 100 mL dry benzene (Caution! Benzene is a carcinogen!) at 0°, very slowly add 10 mmoles α-(3,4-dime-

thoxyphenyl)-4'-bromomethylcinnamonitrile while stirring at 0°, stir at room temperature overnight, add 150 mL water, remove the organic phase, extract the aqueous phase twice with 100 mL portions of diethyl ether, wash the organic layers with saturated NaCl solution, dry over anhydrous magnesium sulfate, evaporate under reduced pressure to give α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile (J.Chem.Eng.Data 1987, 32, 387). Reflux 10 mmoles α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile, 20 mmoles dimethyl sulfate (Caution! Dimethyl sulfate is a carcinogen and acutely toxic!), and 5 g potassium carbonate in 50 mL acetone for 1 h, cool to room temperature, filter, dry the precipitate under vacuum at room temperature overnight, recrystallize from chloroform containing 2-3 drops of 95% EtOH to give α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate (mp 212-215°). Protect solutions from light.)

CHROMATOGRAM
Retention time: k' 0.2791
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: ibuprofen, ketoprofen, mefenamic acid, naproxen, probenecid, valproic acid

KEY WORDS
post-column extraction; post-column reaction

REFERENCE
Kim,M.; Stewart,J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α-phenylcinnamonitrile quaternary ammonium salt as a new fluorescent ion-pair reagent, *J.Liq.Chromatogr.*, **1990**, *13*, 213–237.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin,

mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in the mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 μm Lichrosorb RP 18
Mobile phase: MeOH:water 45:55 containing 1% acetic acid
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 7.63

OTHER SUBSTANCES

Simultaneous: acetaminophen, aspirin, phenacetin, salicylamide

REFERENCE

Nivaud-Guernet,E.; Guernet,M.; Ivanovic,D.; Medenica,M. Effect of eluent pH on the ionic and molecular forms of the non-steroidal anti-inflammatory agents in reversed-phase high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2343–2357.

SAMPLE

Matrix: solutions
Sample preparation: 50 μL Solution + 50 μL pH 7.4 PBS + 100 μL MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 20:80
Flow rate: 1
Injection volume: 50
Detector: UV 220

CHROMATOGRAM

Internal standard: salicylic acid

OTHER SUBSTANCES
Simultaneous: atenolol

KEY WORDS
buffer; Earle's balanced salt solution; salicylic acid is IS

REFERENCE
Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.20 (A), 4.35 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 mm long 5 μm Microsorb-MV C18
Mobile phase: MeOH:water 30:70
Flow rate: 1
Detector: UV 300

REFERENCE
Phillips,C.A.; Michniak,B.B. Transdermal delivery of drugs with differing lipophilicities using azone analogs as dermal penetration enhancers, *J.Pharm.Sci.*, **1995**, *84*, 1427–1433.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.50

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: wine
Sample preparation: Adjust pH of wine to 7-8 with potassium bicarbonate. Remove a 1 mL aliquot and add it to 1 mL 170 mM phenacyl bromide in acetone, add 1 mL 17 mM 18-crown-6 in acetone, add 1 mL acetone, heat in a boiling water bath for 75 min, cool, inject a 10 μL aliquot. (Recrystallize phenacyl bromide from n-heptane.)

HPLC VARIABLES
Guard column: 37-50 μm Bondapak C18/Corasil
Column: 250 × 4 7 μm RP-18 (Merck)
Mobile phase: Gradient. MeOH:water from 35:65 to 85:15 over 20 min.
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11.7

OTHER SUBSTANCES

Extracted: acetic acid, anisic acid, benzilic acid, benzoic acid, butyric acid, caprylic acid, cinnamic acid, citramalic acid, citric acid, enanthic acid, fumaric acid, galacturonic acid, gallic acid, glutaric acid, glycolic acid, glyoxylic acid, p-hydroxybenzoic acid, isocitric acid, α-ketoglutaric acid, lactic acid, malic acid, mandelic acid, phenylacetic acid, propionic acid, protocatechuic acid, pyruvic acid, sorbic acid, succinic acid, tartaric acid, valeric acid, vanillic acid, ascorbic acid

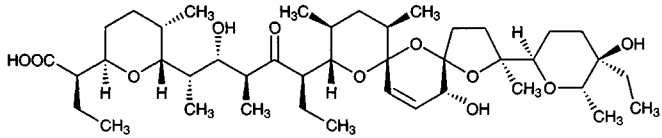
KEY WORDS

derivatization

REFERENCE

Mentasti,E.; Gennaro,M.C.; Sarzanini,C.; Baiocchi,C.; Savigliano,M. Derivatization, identification and separation of carboxylic acids in wines and beverages by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *322*, 177–189.

Salinomycin



Molecular formula: $C_{42}H_{70}O_{11}$
Molecular weight: 751.01
CAS Registry No.: 53003-10-4, 55721-31-8 (sodium salt)
Merck Index: 8488

SAMPLE

Matrix: albumen, eggs, feed, premix, tissue
Sample preparation: Eggs, feed, premix, tissue. 1 g Sample + 15 mL acetone, vortex for 5 min, centrifuge, decant the acetone layer. Re-extract residue (2x), evaporate the combined acetone layers to dryness. Partition the residue between 25 mL aqueous EtOH (water:EtOH 80:20) and 25 mL petroleum ether (50-110°). Evaporate EtOH fraction to dryness, dissolve residue in MeOH, adjust volume to 500 μL. Inject a 50 or 100 μL aliquot. Albumen. 1 g Sample + 15 mL MeOH, extract for 15 min, centrifuge at 1290 × g at -5°. Evaporate to dryness, dissolve residue in MeOH, adjust to a final volume of 500 μL. Inject a 50 or 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Inertsil ODS-2 (Mandel Scientific)
Column: 250 × 3.2 5 μm CSC-Inertsil 150A/ODS-2 (Mandel Scientific)
Mobile phase: MeOH:water:acetic acid 95:5:0.1
Flow rate: 0.5
Injection volume: 50-100
Detector: UV 520 nm following post-column reaction. The column effluent mixed with vanillin reagent pumped at 1 mL/min. The mixture flowed through a Teflon knitted reactor coil (10 m × 0.5 mm i.d., total volume 2 mL) at 95° to the detctor. (Prepare the vanillin reagent as follows. Slowly and carefully add 20 mL concentrated sulphuric acid (95-98%) to 950 mL chilled MeOH, mix well, allow to cool to room temperature. Add 30 g vanillin with constant stirring. Filter solution, degas using vacuum, and store in amber-colored bottle.)

CHROMATOGRAM

Retention time: <10
Limit of detection: 5 ng/g
Limit of quantitation: 10 ng/g

KEY WORDS

post-column reaction